agglutinin remains present for the duration of infection. Of 7 monkeys (*M. philippinensis*) infected with *S. japonicum* 4½ years previously, which still had active infections as demonstrated by positive stool examinations, only 3 showed agglutinins in a titer of 1:128-1:160. The others were negative. It is, of course, possible that the different species of parasites (*S. japonicum* instead of *S. mansoni*) is responsible for these negative tests, but in view of the overlap of the reaction with the trichina infection this is unlikely as an explanation. Six normal monkeys have given negative results.

Discussion. The agglutinin reaction here reported differs from the formation of a precipitate around the cercariae of *Schistosoma mansoni* in that a heat labile factor, possibly complement is necessary for the precipitate reaction(4). It may, however, have some basic similarity to the reaction reported by Vogel and Minning(6) in which a sharply defined homogeneous membrane is formed around the individual cercariae when put in either fresh or inactivated sera. We, however, have not observed any membrane formation during our tests for agglutination. The cross reaction between trichinosis and schistosomiasis was not unexpected for we had previously found that fresh sera from monkeys infected with trichina would form a precipitate around the cercariae of *S. mansoni*(7).

The application of this test to studies in man remains to be explored. Of some seven sera from old but active infections in man with *S. mansoni*‡ or *S. japonicum*, only one *S. mansoni* serum was positive in a dilution of 1/8. We have not tested any sera from humans known to be recently infected.

Summary. The agglutination of living cercariae of *Schistosoma mansoni* by immune sera in high dilutions is reported. This reaction which occurs in heat inactivated as well as in fresh serum also appears in the serum of monkeys recently infected with trichina. The agglutinin develops early in the acute infections and was absent in four of seven chronic schistosome infections.

6. Vogel, H., and Minning, W., *Zbl. Bakter. usw.*, Abt. 1 Orig. 153, 91.

7. Bang, F. B., and Papirmeister, B., unpublished data.

‡ Courtesy of Dr. H. Most, New York University Medical College, N. Y. City.

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Practical Application of a Hemagglutination Reaction in Tuberculosis.* (17813)

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Serological methods in the past have been unsatisfactory in the diagnosis of tuberculosis. Although a variety of immunological techniques had been employed, consistent results from different laboratories were not obtained. Sera from many patients with active tuberculosis failed to react, and in turn, the sera from many normal individuals showed positive tests by these techniques. Difficulties may have arisen from the lack of a suitable antigen of known chemical composition which was capable of reacting with its corresponding antibody. These difficulties were for the most part obviated in 1948 when Middlebrook and Dubos(1) described a specific hemagglutination reaction between sheep erythrocytes treated with a water soluble fraction (rich in polysaccharide) of mammalian tubercle bacilli and the sera of tuber-

* This study was supported by a joint grant-in-aid from United States Public Health Service and the National Tuberculosis Association.

1. Middlebrook, G., and Dubos, R. J., *J. Exp. Med.*, 1948, v88, 521.

culous patients. Because of the difficulty in preparing large quantities of the polysaccharide antigen, it became desirable to find a more easily available supply of antigenic material for a large scale application of this reaction. The studies of earlier workers(2,3) suggested the applicability of old tuberculin as an antigen in tuberculosis serological reactions. One preparation of old tuberculin, Gilliland O. T. (Wyeth), was found by Middlebrook and Dubos(1) to be effective in sensitizing sheep erythrocytes. Scott and Smith(4) have also recently reported the use of another preparation, old tuberculin (Lederle), as a suitable antigen for the hemagglutination test. Of several old tuberculins tested, we noted old tuberculin concentrated approximately 4 times its normal strength, prepared by Lederle Laboratories, to be an excellent antigenic substance and it was therefore used in the studies herein reported.

Materials and methods. The preparation of the materials and the technique of the hemagglutination test were the same as described by Middlebrook and Dubos(1) with the exception of the following points: (a) Instead of the polysaccharide extract of tubercle bacilli, the antigen used for sensitization of the sheep erythrocytes consisted of concentrated old tuberculin (Lederle)† diluted 1:12 in physiological saline with pH adjusted to 6.8-7.0 by addition of dilute NaOH. (b) In the absorption of serum with untreated sheep red cells to remove antibodies unrelated to tuberculous infection, it was found that a single absorption of 2 cc of 1:2 dilution of inactivated serum with 0.2 cc of packed, washed, untreated sheep red cells in a water bath at 37°C for 30 minutes was sufficient. (c) Hyperimmune rabbit serum was employed as a positive control for the antigen-sensitized sheep cells. This serum was prepared by the intravenous injection of 1 cc of heat-killed bovine tubercle bacilli (Ravenel) biweekly for 4 weeks. The bacilli were grown for 10 days in Tween-albumin media(5,6) at 37°C, centrifuged, resuspended in saline and heat-killed at 60°C for 45 minutes. The rabbit serum was inactivated and absorbed in the same manner as the patients' test sera. The human sera to be tested were obtained from in-patients, out-patients, and donors to the blood bank of the Montefiore Hospital and the Montefiore Country Sanatorium. Additional sera were supplied through the kindness of physicians on the staff of the Division of Pulmonary Diseases of this hospital. Sera from patients with syphilis all gave strongly positive Wassermann reactions and were made available by Dr. Widelock of the Serological Laboratories of the New York City Department of Health. As far as could be ascertained skin tests with old tuberculin or any of its products were not performed on patients with active pulmonary tuberculosis.

Results. Results of the hemagglutination tests for tuberculous antibodies in the sera of normal adults, of patients with syphilis, and of patients with non-tuberculous diseases of the chest are summarized in Table I. The sera from patients with syphilis were included because in the past such sera often gave heterologous reactions with the complement fixation test in tuberculosis. Of sera from 110 normal adults, 105 contained no antibodies, 4 had titers of 1:2 and one a titer of 1:4. From the sera of 106 patients with non-tuberculous diseases, 98 gave a negative reaction and 8 showed reactions in serum dilution of 1:2. Thus in this control series of 216 sera, there were 203 with no reactions; 12 with a reaction of 1:2; and one with a reaction in 1:4 dilution of the serum.

In contrast, the results of testing the sera of patients with active tuberculosis, presented

2. Wadsworth, A., Maltaner, F., and Maltaner, E., *J. Immunol.*, 1925, v10, 241.

3. Muether, R. O., and Macdonald, W. C., *J. Lab. and Clin. Med.*, 1945, v30, 411.

4. Scott, N. B., and Smith, D. T., *J. Lab. and Clin. Med.*, 1950, v35, 303.

† Made available through the courtesy of Dr. Henry Piersma of Lederle Laboratories, Pearl River, N. Y.

5. Dubos, R. J., and Davis, B. D., *J. Exp. Med.*, 1946, v83, 409.

6. Dubos, R. J., and Middlebrook, G., *Am. Rev. Tuberc.*, 1947, v56, 334.

TABLE I.
Hemagglutination Tests of Sera from Normal Adults and Patients with Non-tuberculous Diseases.

Diagnosis	No.	Antibody titer: No reaction	1:2	1:4
Normal adults	110	105	4	1
Syphilis	25	23	2	—
Bronchiogenic carcinoma	15	15	—	—
Metastatic lung cancer	15	14	1	—
Bronchiectasis	15	14	1	—
Lung abscess	10	10	—	—
Primary atypical pneumonia	8	8	—	—
Boeck's sarcoid	8	6	2	—
Malignant lymphoma	8	6	2	—
Pneumoconiosis	2	2	—	—
Totals	216	203	12	1

TABLE II.
Hemagglutination Tests of Sera from 201 Patients with Active or Apparently Cured Tuberculosis.

Lesion	No. of patients	Positive: No.	Positive: %	Antibody titer: Lowest	Average	Highest
Pulmonary: Minimal	32	30	93.7	1:8	1:16	1:64
" Moderately advanced	68	60	88.2	1:8	1:32	1:512
" Far advanced	60	57	95.0	1:8	1:32	1:256
Renal or bone	8	8	100.0	1:8	1:64	1:256
Total with active disease	168	155	92.3			
Pulmonary: Apparently cured	33	2		1:2	1:2	1:2

in Table II, demonstrated a high degree of activity. Of 168 patients with active tuberculosis, indicated by the demonstration of tubercle bacilli on staining or on positive culture from the sputa, gastric contents, urine or biopsy specimen, 155 or 92.3% showed positive reactions in titers of 1:8 to 1:512. It is noteworthy that some negative reactions occurred with sera from patients with minimal, moderately, and far advanced pulmonary tuberculosis. In Table II are also presented the results of tests of sera from 33 individuals who have been in good health for at least the past 7 years, but at one time did have active pulmonary tuberculosis. Two sera from this group showed reactions in 1:2 dilution, whereas 31 revealed no reactions. This observation suggests that the presence of these antibodies is in some way related to the activity of the disease.

From a group of 120 tuberculous patients whose sera have been tested in series at monthly intervals, it has been noted that in 10 the antibodies are no longer detectable by this technique. Their disappearance has been correlated with absence of disease activity and failure to demonstrate tubercle bacilli in the sputa or gastric contents on smear or culture. Additional evidence is thus provided that there is a relationship between disease activity and significantly positive reactions in this test. Details of this phase of the study will be described in a subsequent report.

Comment. The data presented confirm and extend the observations of Middlebrook and Dubos(1) on the specific agglutination of sheep erythrocytes sensitized with antigen from tubercle bacilli by sera of tuberculous patients. Other workers(4,7,8) have also recently reported successful use of this method in serological studies of tuberculosis. This hemagglutination reaction has become more

7. Gernez-Rieux et Tacquet, A., *Bull. Acad. Nat. Med.*, 1949, 556.

8. Sohier, R., *Ann. Inst. Pasteur*, 1950, v78, 283.

practical with the demonstration that concentrated old tuberculin (Lederle) can be substituted as the antigen for sensitization of sheep erythrocytes instead of the polysaccharide fraction previously described(1). The heat stable polysaccharide fraction isolated from mammalian tubercle bacilli is undoubtedly contained in the old tuberculin preparation. Heterologous reactions were noted in approximately 6% of sera from non-tuberculous patients or normal adults in the present series, but these reactions are of lower titer than those obtained from patients with active tuberculosis. Because of the cross reactions, it appears probable that only titers of 1:8 or higher are indicative of tuberculous infection. The potential value of the test is suggested by the fact that 92% of tuberculous patients' sera showed positive reactions in titers of 1:8 or higher, whereas in a precipitin test for carbohydrate antibodies in human tuberculosis recently reported, 50% positive reactions were found(9).

From these observations it seems that this hemagglutination test apparently not only provides a valuable diagnostic tool, but unlike the tuberculin skin test, appears to indicate the presence of tuberculous activity and therein lies its greatest value.

Summary. The sera of 216 normal adults and patients with syphilis or non-tuberculous chest diseases were tested for tuberculous antibodies by the hemagglutination reaction: 203 showed no reactions; 12 had positive reactions in serum dilution of 1:2; and one in dilution of 1:4. The sera of 168 patients with active tuberculosis showed 92% positive reactions in dilutions ranging from 1:8 to 1:512. Sera from 33 apparently cured tuberculous individuals revealed no reactions in 31, and positive reactions in 2 in a serum dilution of 1:2. This hemagglutination reaction is apparently a relatively specific test for active tuberculosis.

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Separation of Pertussal Toxin.* (17814)

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Toxic extracts have been prepared from *Hemophilus pertussis* by a variety of methods(1-9). Pertussal toxin is lethal for mice, guinea pigs and rabbits and is dermonecrotic. It is heat-labile, easily detoxified by formaldehyde, and antigenic. Rabbit pertussal antitoxic sera will neutralize the toxin in multiple proportions and induce passive immunity in guinea pigs(10) and in rabbits and mice(11). The purpose of this investigation is to report methods for the extraction and separation of pertussal toxin from a variant toxin-producing strain of *H. pertussis*. The toxin can be extracted from dried *H. per-*

* Aided by a grant from Lederle Laboratories Division, American Cyanamid Co.

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